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(19)



Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number:

0 385 433
A2

(12)

EUROPEAN PATENT APPLICATION

(21) Application number: 90103896.8

(51) Int. Cl.⁵: C07K 1/04, B01J 19/00

(22) Date of filing: 28.02.90

(30) Priority: 28.02.89 CS 1280/89

(43) Date of publication of application:
05.09.90 Bulletin 90/36(84) Designated Contracting States:
DE FR GB IT SE

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EP 0 385 433 A2

(54) Method and apparatus for the continuous polymer synthesis on a solid carrier.

(57) The invention relates to a novel method and apparatus for the solid-phase synthesis of polymers such as polypeptides wherein an elongated, preferably band-like carrier (1) is used. The attachment of the reactants is realized by successive introduction of the carrier (1) into the appropriate baths. This method allows continuous preparation of polypeptides, polynucleotides, etc.

The apparatus comprises a solid, insoluble, elongated carrier (1) comprising functional groups and an arrangement of successive reaction zones (5, 7) and processing zones (4, 6, 8) of a sequence identical with that of the reaction and processing steps of the process sequence of the respective synthesis, and comprises means (2a, 2b) for successively contacting the carrier (1) with the reaction and processing liquids.

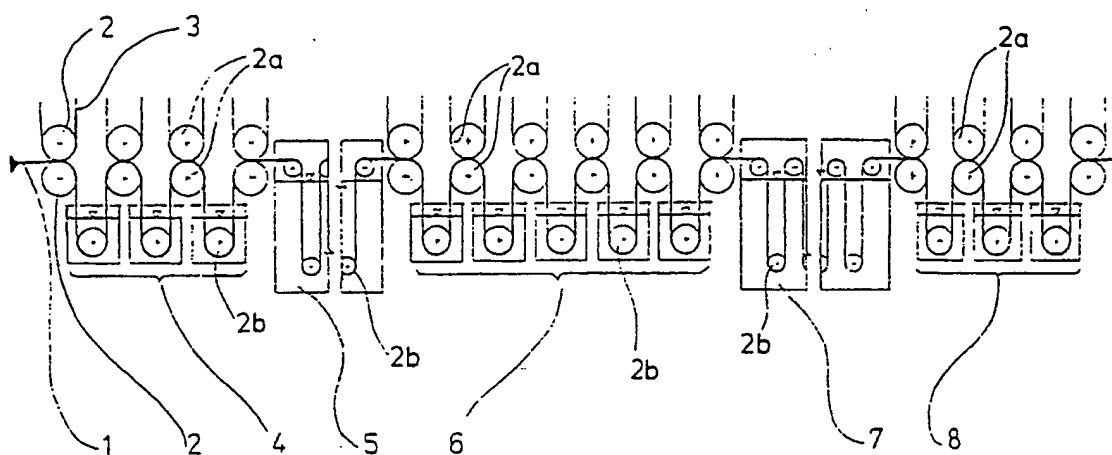


FIG. 1

METHOD AND APPARATUS FOR THE CONTINUOUS POLYMER SYNTHESIS ON A SOLID CARRIER

This invention relates to a method, an apparatus and a carrier for the continuous synthesis of oligomer and macromolecular organic compounds and particularly of biopolymers such as oligo- and polysaccharides, oligo- and polynucleotides, and in particular oligo- and polypeptides on a solid carrier.

Peptides are prepared by classical methods in solution (Houben-Weyl, Methoden der Organischen Chemie, Synthese von Peptiden (E. Wünsch ed.) Thieme-Verlag, Berlin 1974) as well as by solid phase synthesis developed by Merrifield (for a recent review see e.g. Stewart, J.M., and Young, J.D., Solid Phase Peptide Synthesis, Freeman, San Francisco 1969; second edition 1985). The solid phase method has the advantage of a high speed because during the synthesis the intermediates are isolated simply by collecting the solid carrier and washing it with solvents. This method has been shown to be suitable for automation (see e.g. Merrifield, R.B., Stewart, J.M., and Jernberg, N., Apparatus for the automated synthesis of peptides, US-A-3 531 258; Brunfeldt, K., Roepstorff, P., and Halstrom, J., Reaction system, US-A-3 557 077; Kubodera, T., Hara, T., and Makabe, H., Apparatus for synthesis of peptides or the like organic compounds, US-A-3 647 390; Won Kil Park and Regoli, D., System for the solid phase synthesis, US-A-3 715 190; Verlander, M.S., Fuller, W.D., and Goodman, M., Rapid, large scale, automatable high pressure peptide synthesis, US-A-4 192 798; Hamill, B.J., Apparatus for the chemical synthesis of oligonucleotides, US-A-4 728 502; Neimark, J., and Briand, J.-P., Semi-automatic, solid-phase peptide multi-synthesizer and process for the production of synthetic peptides by the use of multi-synthesizer, US-A-4 748 002; Bridgham, J., et al., Automated polypeptide synthesis apparatus, US-A-4 668 476; Saneii, H.H., Solid phase synthesizer, US-A-4 746 490).

Some other chemical and physico-chemical processes have been successfully modified for continuous operation (colouring of textiles (US-A-4 863 154), developing of films, etc.) and automated. Devices serving for similar purposes are used e.g. in glue spreading onto tapes (FR-A-2 627 108), cloth and paper impregnation (FR-A-2 612 086, US-A-4 863 085), belt driers (US-A-3 931 684), electrographical devices (US-A-3 783 827), or in the application of several liquid layers onto textiles (DE-AI-3 414 504).

Obviously, continuous performance of peptide synthesis would enable continuous production of these highly sophisticated organic compounds. Up to now, no attempts on the continuous modification of peptide synthesis have been made, mostly because no suitable continuous method and carriers have been available.

It is the object of this invention to provide a method, an apparatus, and carriers for the continuous synthesis of oligomeric and polymeric organic compounds and particularly of biopolymers, such as oligo- and polysaccharides, oligo- and polynucleotides, and in particular oligo- and polypeptides which are suitable for automation.

This object is achieved according to the claims. The dependent claims relate to preferred embodiments.

The method of the present invention for the continuous synthesis of oligomeric and polymeric organic compounds, particularly of biopolymers, and preferably of polypeptides and polynucleotides, is characterized in that

- a solid, insoluble, elongated carrier is used comprising functional groups serving for anchoring the first reaction component of the process sequence of the respective synthesis either directly or by means of a spacer,

and

- the individual reaction and processing steps are carried out in successive zones of a sequence identical with that of the reaction and processing steps of the process sequence moving in one direction given by the longest dimension of the carrier.

In accordance with a preferred embodiment, all reaction and processing steps of the process sequence are carried out at the same time in different parts of the carrier.

In accordance with another preferred embodiment, the carrier is successively subjected to the reaction and processing steps of the process sequence.

It is preferred to move the carrier through the successive zones, preferably with a predetermined, constant speed.

Another preferred embodiment is characterized in that an open- or closed-loop carrier is used the length of which is at least such as to correspond to the total residence time necessary for all reaction and processing steps of the process sequence for a given moving speed.

For obtaining some or all intermediates and eventually also the desired final product, the synthesis process is stopped at a stage where these compounds corresponding to the respective series of reaction

steps of the process sequence have been formed in the corresponding parts of the carrier, and these compounds are removed from the carrier either continuously or in a batch-like manner from the respective parts of the carrier.

The final product is preferably removed from the carrier after completion of the total process sequence in all parts of the carrier, preferably in a batch-like manner.

The process sequence comprises all reaction and processing (e.g. washing) steps in the necessary order which are required for the synthesis of a given polymer product.

In accordance with the general concept of this invention all the reactions needed for the synthesis of the given polymer proceed at the same time in different parts of the carrier. The carrier preferably is a planar insoluble material containing suitable functional groups for anchoring the reaction component of the process sequence, i.e. for example the first amino acid in the case of peptide synthesis and is successively brought into contact with the reagent used for the sequential solid-phase synthesis.

More specifically, the method according to this invention represents a modified form of the solid phase synthesis enabling continuous production.

The apparatus of this invention for the continuous synthesis of oligomeric and polymeric organic compounds, particularly for carrying out the above-defined method, comprises

- a solid, insoluble, elongated carrier comprising functional groups serving for anchoring the first reaction component of the process sequence, and

- an arrangement of successive reaction and processing zones of a sequence identical with that of the reaction and processing steps of the process sequence of the respective synthesis, and comprising means for successively contacting the carrier with the liquids of the reaction and processing zones.

In accordance with preferred embodiments, the apparatus comprises an open- or closed-loop carrier the length of which is at least such that it corresponds to the total residence time necessary for all reaction and processing steps of the process sequence for a given moving speed, and the carrier path length of the individual reaction and processing steps corresponds to the residence time necessary for the respective reaction or processing steps for the given moving speed. The means for contacting the carrier with the reactant and processing liquids are preferably rolls or rollers defining a required path length in the respective liquid vessels.

The solid carrier is advantageously in the form of a band. The carrier band is preferably led, e.g. by means of a series of rollers, through the appropriate reagents and washing solvents so that the individual reactants, e.g. amino acids, are stepwise bonded, and in the last step the synthesized polymer, e.g. peptide, is cleaved off. The band with the recovered original functional groups can be returned to the beginning of the whole sequence of operations, and the whole cycle may be repeated again, if suitable carriers and spacers are used. Unlike in the classical arrangement of solid phase synthesis, in which the individual steps have to be performed subsequently with the whole carrier, in the method according to this invention all the synthetic steps proceed simultaneously, at locally different sites. Another advantage is that the product is obtained in a continuous process instead of a single batch process.

The method may be used for the most important strategies of solid phase peptide synthesis, i.e. the so-called Boc and Fmoc strategies. Nevertheless, the latter method Fmoc, using the base-cleavable N-amino-protecting group, allows a substantially easier arrangement: less washing steps are required, and already known spacers, enabling acid-catalyzed liberation of the final peptide may be employed. Depending on the character of the carrier, the use of other protecting groups is also possible. Thus, e.g. in such a synthesis using a relatively polar cotton band as the carrier, protection was achieved with the o-nitrobenzenesulfonyl group for which a nonpolar carrier of polystyrene type is not suitable. The synthesis can be monitored visually by an acid-base indicator (CS patent application PV 209-88).

The method and apparatus according to the invention are applicable especially for manufacturing purposes, the desired final product, such as a polypeptide or polynucleotide, being produced continuously in amounts corresponding to the capacity and size of the carrier used.

The continuous solid phase synthesis may also be adjusted for small-scale preparation of many biopolymers such as polypeptides and polynucleotides: in such cases the carrier is preferably a thread which is successively introduced into solutions of the corresponding activated amino acids, and when the synthesis has ended the whole carrier is subjected to the isolation procedure. In this way, a whole series of peptides of various length may be prepared, their number being limited by the capacity of the individual segments.

In many cases the desired end product is a macro-molecule which is directly bonded to the polymeric carrier. If the form of this carrier is suitable, the synthesis may be performed directly on the carrier which has been only processed mechanically or chemically.

The (condensation) reactions may be accelerated either by working in an ultrasonic bath or by leading the carrier band over a heated roller. Washing of the carrier is facilitated by pressing between two rollers and passing through, or moistening with the corresponding solvent.

Suitable carriers are polymeric materials containing suitable functional groups, such as cellulose (e.g. paper or cotton bands), modified polystyrene, polytetrafluoroethylene (teflon), polyamides, modified polypropylene, functionalized glass, etc.

Examples for suitable carriers which have been tested experimentally are cotton tissues, glass fibres modified by aminopropyl groups, or polypropylene membranes modified by hydroxypropylacrylate (product of MilliGen). Peptides obtained on all these carriers were analyzed thoroughly and were found identical. However, cotton is superior because it has a most favourable combination of good mechanical and physico-chemical properties.

An example of the arrangement of one synthesizer segment of the apparatus according to the present invention is shown in Figure 1. The carrier 1 coming e.g. from the preceding synthetic step is pressed between a pair of rollers 2 which removes most of the liquid brought from the preceding step. The removal is facilitated by simultaneous introduction of another porous material 3, such as paper or textile material, between the rollers. The carrier advances into the washing bath 4 which comprises 3 stages and represents a processing zone, and is pressed again between rollers 2a. This operation is repeated several times, and then the carrier 1 is introduced into a solution of reagent removing the N-amino-protecting group in a reaction zone 5. The duration of the contact of the carrier with this solution is determined by the length of the pathway through the bath. This is achieved by using a system of pulleys 2b which are also provided in the processing zones 4, 6 and 8, and in the reaction zones 5 and 7. From the bath of zone 5 the carrier proceeds further into a series of washing baths, representing a processing zone 6, and then into a vessel containing the activated protected amino acid (e.g. the symmetrical anhydride) in the reaction zone 7. Here the path length is again adjusted by means of the pulleys 2b so as to achieve the residence time required for completion of the condensation reaction. The carrier 1 then enters again the system of rollers 2a and washing baths in the processing zone 8, and after washing it can undergo the next cycle in which another amino acid is attached and which is performed in a segment analogous to the above-mentioned one. All the baths must be continuously regenerated so as to keep a suitable concentration of reagents and avoid accumulation of contaminants.

In principle, the same equipment may be used e.g. for the cleavage of protecting groups and washing, and the carrier is introduced into separated vessels only for the condensation reaction. This applies particularly to the above-mentioned embodiment with a thread-like carrier.

A more simple arrangement of a synthesizer segment of an apparatus according to this invention is shown in Figure 2. In this case, the carrier 1 is moistened with the solvent or a solution containing the activated component (e.g. FMOC-amino acid in a solvent, or another reagent solution (piperidine/DMF)). Because of the high soaking capacity of the carriers used, the volume of soaked liquid is sufficient for successful execution of the corresponding reaction (e.g. mass of dimethylformamide per mass of the fabric: glass 0.4 g/g, polypropylene 1.0 g/g, cotton 1.1 g/g). The carrier 1 is guided and pressed between pairs of rollers 9; the washing zones 4 are again provided after the reaction zones. The solvent (DMF) is supplied by means of a link system 10.

This version is more economical concerning the solvent consumption, and, moreover, it guarantees high quality of the solvents because fresh solvent is continuously supplied.

For practical purposes it should be realized that even with relatively small dimensions of the carrier (e.g. width 3 cm), low substitution (3 $\mu\text{mol/cm}$) and low band velocity (5 cm/min, i.e. 3m/h) the equipment is capable of producing e.g. 65 g of a peptide of a molecular mass of 1000 during 24 h. This fact allows the use of carriers with extremely low substitution (e.g. modified glass fabrics).

The method and apparatus according to the present invention are illustrated by the following examples.

50 Example 1

A cotton band (width 3 cm, length 280 cm, specific mass 23,1 mg/cm) was shaken with a mixture of trifluoroacetic acid (25 ml) and dichloromethane (75 ml) for 15 min, washed successively with dichloromethane (3 x 100 ml), a 10 % solution of diisopropylethylamine in dichloromethane (2 x 100 ml) and dichloromethane (3 x 100 ml), and dried in vacuo. The band was then shaken with a mixture of t-butyloxycarbonylglycine (4.4 g), dicyclohexylcarbodiimide (5.15 g), dimethylaminopyridine (3.05 g), and dimethylformamide (100 ml) for 4 h at room temperature. After washing with dimethylformamide (3 x 100 ml), ethanol (3 x 100 ml) and dichloromethane (3 x 100 ml) and drying, the capacity of the carrier was 3.1

μmol/cm.

Example 2

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Estimation of the carrier capacity.

A sample of the carrier (1 cm) was shaken with a 25 % solution of trifluoroacetic acid in dichloromethane for 15 min and washed successively with dichloromethane (3 x), a 10 % solution of diisopropylethylamine in dichloromethane, and with dichloromethane. It was then shaken with fluorenylmethoxycarbonylglycine, dicyclohexylcarbodiimide and N-hydroxybenzotriazole in the presence of a small amount of bromophenol blue until the blue colour of the carrier disappeared. The sample was then washed with dimethylformamide (3 x) and shaken with a 20 % solution of piperidine in dimethylformamide for 15 min. The piperidine solution was separated, and made up to 50 ml with the dimethylformamide employed for the above washing of the sample; then the extinction of the solution at 301 nm was read. The carrier capacity was then calculated from the extinction value and the known extinction coefficient of the adduct of fulvene with piperidine (7 800).

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Example 3

Synthesis of des-glycyl-methionine-enkephalin.

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A cotton band (20 cm) with bound t-butyloxycarbonylglycine was shaken with a 25 % solution of trifluoroacetic acid in dichloromethane for 15 min and washed successively with dichloromethane (3 x), a 10 % solution of diisopropylamine in dichloromethane (2 x), and dichloromethane (3 x). It was then shaken overnight with 2,4,5-trichlorophenyl-3-(4-fluorenylmethyl-oxycarbonylmethionyl-oxymethylphenoxy)-propionate (3 mmol) and N-hydroxybenzotriazole (3 mmol) in dimethylformamide (15 ml) at room temperature. Then the carrier substitution was determined by removal of the Fmoc group (1 μmol/cm), and the carrier was acetylated with a mixture of acetic anhydride (5 ml) and pyridine (10 ml) for 30 min at room temperature. The carrier was washed with dimethylformamide (3 x) and dichloromethane (3 x). The cotton band was subjected to three cycles of solid phase peptide synthesis, described in Example 5 using fluorenylmethoxycarbonylphenylalanine, fluorenylmethyl oxycarbonylglycine and t-butyloxycarbonyl-t-butyltyrosine as protected amino acids. The carrier was then introduced into a solution consisting of 50 % of trifluoroacetic acid, 5 % of dimethylsulfide and 45 % of dichloromethane. After 2 h the band was washed with the same solution, and the combined eluates were concentrated in vacuo to a small volume. The product was precipitated with absolute ether, separated, dried, and dissolved in 1 M acetic acid. The solution was then filtered and freeze-dried to give 30 mg of the crude product which was purified by high pressure liquid chromatography on a column (Vydac C18, 250 x 10 mm). The chromatography furnished 8.6 mg of the product which was pure according to high pressure liquid chromatography (column Spherisorb ODS II 250 x 4 mm, k = 6.4; 75 % methanol in 0.05 % trifluoroacetic acid) as well as thin-layer chromatography in four systems, and electrophoresis at two pH values.

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Amino acid analysis: Gly 1.00, Met 0.92, Tyr 0.89, Phe 0.96.

Mass spectrometry: 517 (M + H).

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Analysis for C ₂₅ H ₃₂ N ₄ O ₆ S • CF ₃ COOH • 2H ₂ O (666.7):			
calculated:	48.64 % C,	5.59 % H,	8.40 % N;
found:	48.87 % C,	5.32 % H,	8.56 % N.

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Example 4

Synthesis of methionine-enkephalin.

The synthesis was executed as described in Example 3 except that the condensation of protected glycine was repeated twice.

Yield 15,6 mg of product, pure according to high-pressure liquid chromatography (column of Spherisorb ODS II 250 x 4 mm, $k = 5.8$, 75 % methanol in 0.05 trifluoroacetic acid), thin-layer chromatography in four solvent systems, and electrophoresis at two pH values.

Amino acid analysis: Gly 2.00, Met 0.90, Tyr 0.86, Phe 0.99.

Mass spectrometry: 574 (M + H).

Analysis for $C_{27}H_{35}N_5O_7S \cdot CF_3COOH \cdot 2H_2O$ (723.7):			
calculated:	48.13 % C,	5.57 % H,	9.68 % N;
found:	48.58 % C,	5.38 % H,	9.42 % N.

Example 5

Cycle employed for attachment of an amino acid residue using fluorenylmethyloxycarbonyl protecting group.

The carrier is successively introduced into the following solutions and solvents:

1. ethanol
2. dichloromethane
3. dichloromethane
4. dichloromethane
5. dimethylformamide
6. dimethylformamide
7. dichloromethane
8. dichloromethane
9. fluorenylmethyloxycarbonylamino acid, dicyclohexylcarbodiimide, N-hydroxybenzotriazole - all 0.2 M solutions in dimethylformamide
10. dimethylformamide
11. dimethylformamide
12. ethanol.

After passage through each bath the carrier is introduced between rotating rollers, together with a strip of filtering paper which absorbs the solvent which has been pressed out. The carrier is then introduced into the next bath. The immersion time in the individual baths is 2 min except for bath No. 4 in which the carrier is immersed for 20 min, and bath No. 9 in which the time depends on the disappearance of the blue colour of the indicator (bromophenol blue).

Example 6

Cycle employed for attachment of an amino acid residue using the t-butyloxycarbonyl protecting group.

The carrier is successively introduced into the following solutions and solvents:

1. ethanol
2. dichloromethane
3. dichloromethane
4. 45 % trifluoroacetic acid and 5 % anisol in dichloromethane

5. dichloromethane
6. dichloromethane
7. dichloromethane
8. 10 % diisopropylamine in dichloromethane
- 5 9. dichloromethane
10. dichloromethane
11. dimethylformamide
12. dimethylformamide
13. t-butyloxycarbonylamino acid, dicyclohexylcarbodiimide, N-hydroxybenzotriazole - all 0.2 M
- 10 solutions in dimethylformamide
14. dimethylformamide
15. dimethylformamide
16. ethanol.

After passage through each bath, the carrier passes between rotating rollers together with a strip of
 15 filtrating paper which absorbs the solvent which has been pressed out. The carrier is then introduced into
 the next bath. The immersion time in the individual baths is 2 min except for bath No. 4 in which the carrier
 is immersed for 20 min, bath No. 8 (6 min), and bath No. 13 in which the time depends on the
 disappearance of the blue colour of the indicator (bromophenol blue).

20 Example 7

Synthesis of bound methionine-enkephalin.

25 A band that had been processed as described in Example 1 was successively acylated with
 fluorenylmethyloxycarbonyl-protected methionine, phenylalanine, glycine, glycine and o-nitrophenylsulfenyl-
 tyrosine, using the procedure described in Example 5. The o-nitrophenylsulfenyl protecting group was
 removed by treatment with 7.5 M hydrogen chloride in methanol (20 min).

30 The enkephalin content as determined by amino acid analysis was 1.6 $\mu\text{mol/cm}$ of the carrier.

Example 8

35 Synthesis of methionine-enkephalin.

A piece of a polypropylene membrane modified with hydroxypropylacrylate (3 x 3 cm) was acylated
 with Fmoc-Gly in the same manner as described in Example 1. By cleavage of the Fmoc groups the
 40 substitution was found to be 0.1 mmol/g.

The synthesis of the peptide was performed as described in Example 4. After the same treatment, a
 peptide was obtained which could not be distinguished from that prepared in Example 4.

45 Example 9

Synthesis of methionine-enkephalin.

50 A piece of fibre glass modified with aminopropyl groups (25 x 6 cm, 1 g, 1.7 μmol) was acylated
 overnight with 2,4,5-trichlorophenyl-3-(4-fluorenylmethyl-oxycarbonylmethionyl-oxymethylphenoxy)-pro-
 pionate (0.05 mol) and N-hydroxybenzotriazole (0.05 mmol) in dimethylformamide (0.4 ml) at room
 temperature. The carrier was then washed with dimethylformamide (3 x) and subjected to three cycles of
 solid phase peptide synthesis, described in Example 5 using fluorenylmethyloxycarbonylphenylalanine,
 55 fluorenylmethyloxycarbonylglycine and t-butyloxycarbonyl-O-t-butyltyrosine as protected amino acids. The
 carrier was then introduced into a solution consisting of 95 % of trifluoroacetic acid and 5 % of dimethyl
 sulfide. After 2 h the band was washed with the same solution, and the combined eluates were evaporated
 in vacuo, dissolved in 3 M acetic acid and lyophilized. The product was dissolved in 1 M acetic acid, and

the solution was applied on a column (Vydac C18 250 x 4 mm). The chromatography furnished 0.3 mg of the product which was pure according to high pressure liquid chromatography and identical with the above-described methionine-enkephalin.

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Example 10

Synthesis of model peptides.

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A cotton band that had been acylated with t-butyloxycarbonylalanine as described in Example 1 was subjected to the synthesis cycles described in Example 6, and successive attachment of t-butyloxycarbonyl-protected amino acids was performed. The obtained modified carrier was then subjected to either alkaline hydrolysis (0.1 M-NaOH, 2 h at room temperature) or ammonolysis (saturated ammonia solution in methanol, 4 h at room temperature).

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The following peptides were obtained by this procedure:

H-Pro-Leu-Gly-Ala-OH

H-Pro-Leu-Gly-Ala-NH₂

H-Leu-Pro-Gly-Ala-OH

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H-Leu-Pro-Gly-Ala-NH₂.

However the described method of removing the peptide from the carrier is accompanied by formation of several side products due to the glycine in position 3.

25 Claims

1. A method for the continuous synthesis of oligomeric and polymeric organic compounds, particularly of biopolymers and preferably of polypeptides and polynucleotides, characterized in that

- a solid, insoluble, elongated carrier is used comprising functional groups serving for anchoring the first reaction component of the process sequence of the respective synthesis either directly or by means of a spacer,
and

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- the individual reaction and processing steps are carried out in successive zones of a sequence identical with that of the reaction and processing steps of the process sequence moving in one direction given by the longest dimension of the carrier.

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2. The method according to claim 1, characterized in that all reaction and processing steps of the process sequence are carried out at the same time in different parts of the carrier.

3. The method according to claims 1 and 2, characterized in that the carrier is successively subjected to the reaction and processing steps of the process sequence.

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4. The method according to claims 1 to 3, characterized in that the carrier is moved through the successive zones, preferably with a predetermined, constant speed.

5. The method according to claims 1 to 4, characterized in that a planar, band-shaped, or thread-like carrier is used.

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6. The method according to claims 1 to 5, characterized in that a carrier is used made of a cellulose material, such as cotton fabric or paper, modified polystyrene, polytetrafluoroethylene, polyamide, modified polypropylene, or functionalized glass, such as modified glass fibers or modified fibre glass.

7. The method according to claims 1 to 6, characterized in that an open- or closed-loop carrier is used the length of which at least corresponds to the total residence time necessary for all reaction and processing steps of the process sequence for a given moving speed.

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8. The method according to claims 1 to 7, characterized in that the synthesis process is stopped at a stage where a series of intermediate compounds and eventually also the derived final product corresponding to the respective series of reaction steps of the process sequence have been formed in the corresponding parts of the carrier, and these compounds are removed from the carrier either continuously or in a batch-like manner from the respective parts of the carrier.

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9. The method according to claims 1 to 8, characterized in that the final product is removed from the carrier after completion of the total process sequence in all parts of the carrier, preferably in a batch-like manner.

10. An apparatus for the continuous synthesis of oligomeric and polymeric organic compounds,

particularly for carrying out the method according to claims 1 to 9, comprising

- a solid, insoluble, elongated carrier (1) comprising functional groups serving for anchoring the first reaction component of the process sequence,

and

- 5 - an arrangement of successive reaction and processing zones (4, 5, 6, 7, 8) of a sequence identical with that of the reaction and processing steps of the process sequence of the respective synthesis, and comprising means (2a, 2b) for successively contacting the carrier (1) with the liquids of the reaction and processing zones (4, 5, 6, 7, 8).

11. The apparatus according to claim 10, comprising means for continuously moving the carrier (1) 10 through the reaction and processing zones (4, 5, 6, 7, 8) with a predetermined, constant speed.

12. The apparatus according to claims 10 and 11, characterized in that it comprises an open- or closed-loop carrier (1) the length of which at least corresponds to the total residence time necessary for all reaction and processing steps of the process sequence for a given moving speed, and the carrier path length of the individual reaction and processing steps corresponds to the residence 15 time necessary for the respective reaction or processing step for the given moving speed.

13. The apparatus according to claims 10 to 12, characterized in that the carrier (1) is planar, band-shaped or thread-like.

14. Carrier for use in the method according to claims 1 to 9 and/or in the apparatus according to claims 10 to 13, characterized in that it is made of a cellulose material, such as cotton fabric or paper, modified 20 polystyrene, polytetrafluoroethylene, polyamide, modified polypropylene, or functionalized glass, such as modified glass fibers or modified fibre glass.

15. Use of the apparatus according to claims 10 to 13 and/or of the carrier according to claim 14 for and/or application of the method according to claims 1 to 9 to peptide or polynucleotide synthesis.

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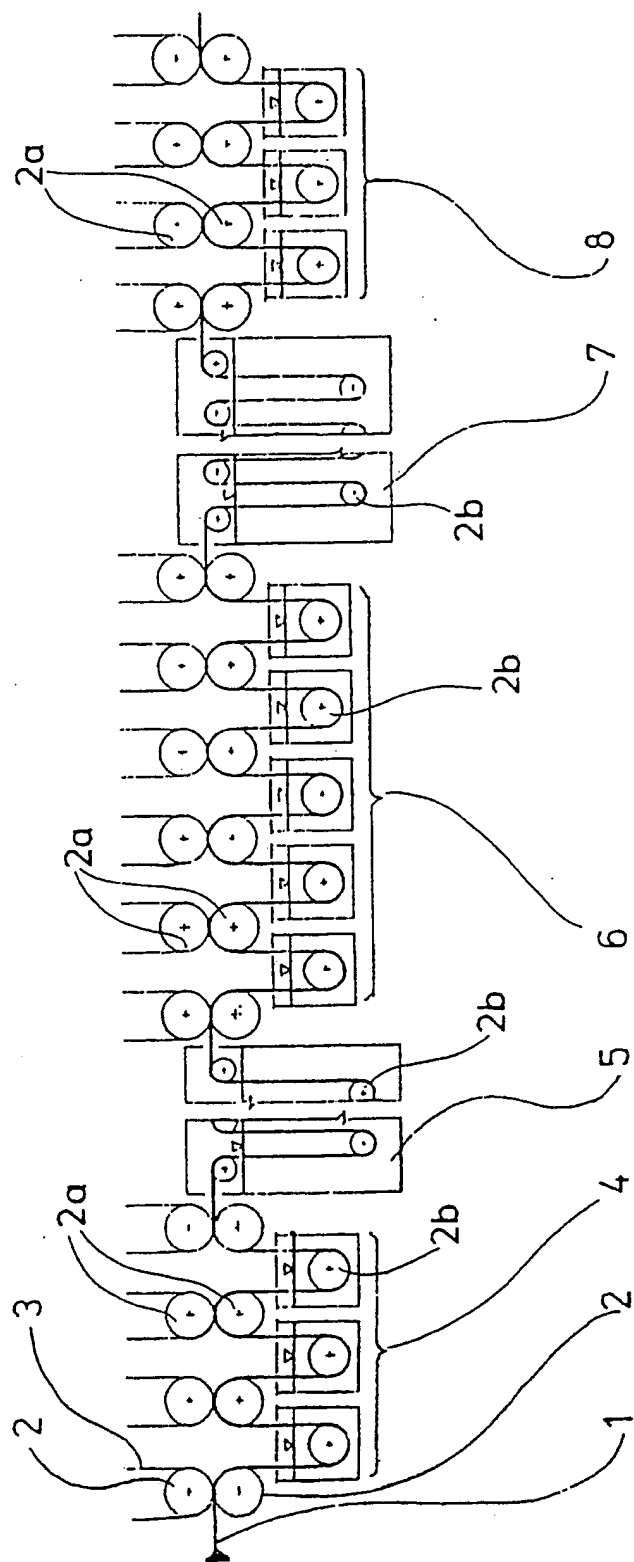


FIG.1

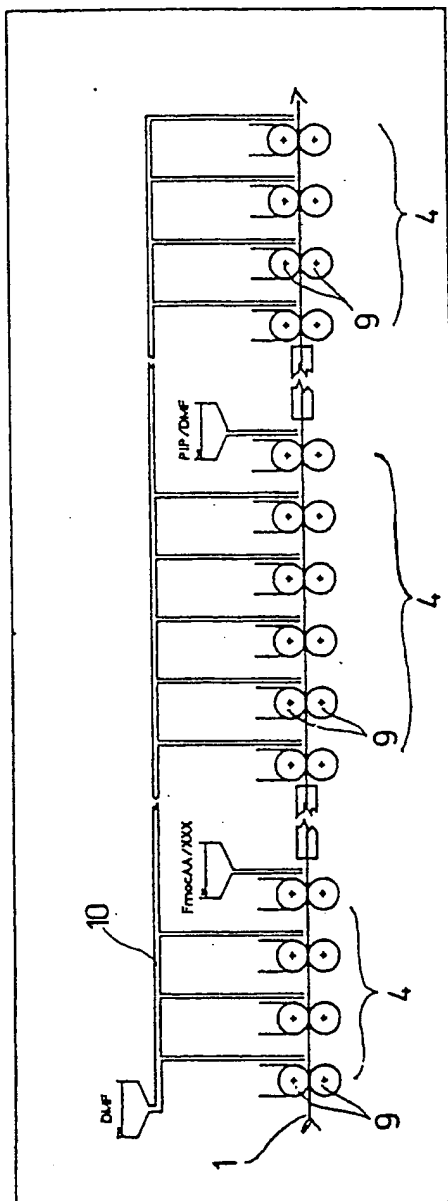


FIG. 2

(19)



Europäisches Patentamt
European Patent Office
Office européen des brevets



(11) Publication number:

0 385 433 A3

(12)

EUROPEAN PATENT APPLICATION

(21) Application number: 90103896.8

(51) Int. Cl.⁵: **C07K 1/04, B01J 19/00,
B01J 19/22**

(22) Date of filing: 28.02.90

(30) Priority: 28.02.89 CS 1280/89

(43) Date of publication of application:
05.09.90 Bulletin 90/36

(84) Designated Contracting States:
DE FR GB IT SE

(89) Date of deferred publication of the search report:
14.08.91 Bulletin 91/33

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EP 0 385 433 A3

(54) Method and apparatus for the continuous polymer synthesis on a solid carrier.

(57) The invention relates to a novel method and apparatus for the solid-phase synthesis of polymers such as polypeptides wherein an elongated, preferably band-like carrier (1) is used. The attachment of the reactants is realized by successive introduction of the carrier (1) into the appropriate baths. This method allows continuous preparation of polypeptides, polynucleotides, etc.

The apparatus comprises a solid, insoluble, elongated

carrier (1) comprising functional groups and an arrangement of successive reaction zones (5, 7) and processing zones (4, 6, 8) of a sequence identical with that of the reaction and processing steps of the process sequence of the respective synthesis, and comprises means (2a, 2b) for successively contacting the carrier (1) with the reaction and processing liquids.

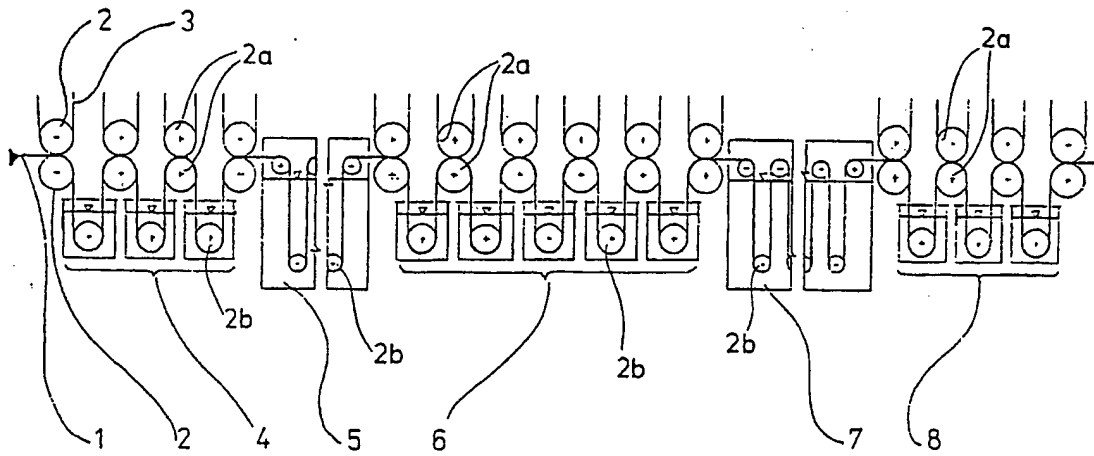


FIG.1



European
Patent Office

EUROPEAN SEARCH REPORT

Application Number

EP 90 10 3896

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
X	DE-A-1 593 880 (HOECHST) * Whole document, especially drawing no. 3; figure 1 * -----	1-15	C 07 K 1/04 B 01 J 19/00 B 01 J 19/22
A	EP-A-0 260 634 (CBF) * Whole document, especially page 1, line 48 - page 3, line 20 * -----	1	
			TECHNICAL FIELDS SEARCHED (Int. Cl.5)
			C 07 K B 01 J
The present search report has been drawn up for all claims			
Place of search		Date of completion of search	Examiner
The Hague		10 May 91	MASTURZO P.
<div>CATEGORY OF CITED DOCUMENTS</div> <div><div>X: particularly relevant if taken alone Y: particularly relevant if combined with another document of the same category A: technological background O: non-written disclosure P: intermediate document T: theory or principle underlying the invention</div><div>E: earlier patent document, but published on, or after the filing date D: document cited in the application L: document cited for other reasons ----- &: member of the same patent family, corresponding document</div></div>			

[54] PROCESS AND DEVICE FOR MEASURING THE TWIST OF A RUNNING, ELONGATE TEST BODY

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[21] Appl. No.: 337,521

[22] PCT Filed: Jun. 29, 1988

[86] PCT No.: PCT/CH88/00116

§ 371 Date: Feb. 9, 1989

§ 102(e) Date: Feb. 9, 1989

[87] PCT Pub. No.: WO89/00215

PCT Pub. Date: Jan. 12, 1989

[30] Foreign Application Priority Data

Jul. 6, 1987 [CH] Switzerland 02565/87

[51] Int. Cl.⁵ G01N 21/86

[52] U.S. Cl. 250/571; 356/429

[58] Field of Search 57/264, 265; 73/159, 73/160; 250/561, 571, 572; 356/429, 430

[56] References Cited

U.S. PATENT DOCUMENTS

3,557,377 1/1971 Raasch 250/571

FOREIGN PATENT DOCUMENTS

1560540 6/1970 Fed. Rep. of Germany .
3628654A1 3/1988 Fed. Rep. of Germany .
50-30184 9/1975 Japan .
5030184 9/1975 Japan .

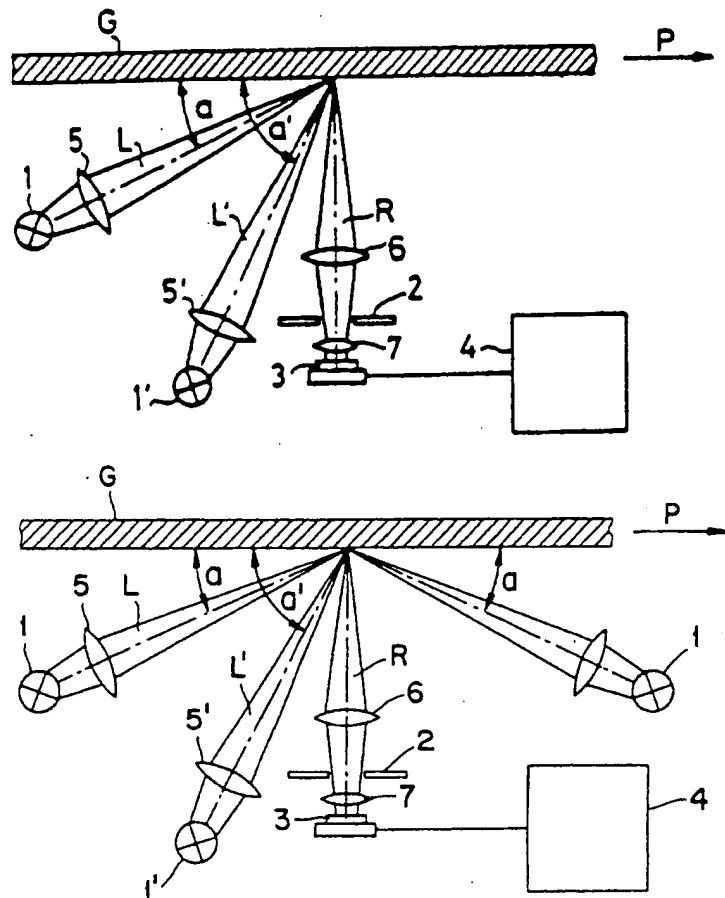
Primary Examiner—David C. Nelms

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[57] ABSTRACT

A test body is illuminated by at least one light source obliquely to its direction of travel, and the reflected light is imaged onto a diaphragm and fed to a photoelectric receiver. Its signal is investigated, in an evaluating unit, for periodicities which are caused by irregularities included in the test body as a result of the twist and the wavelength of which represents a measure of the twist. A rapid and precise measurement of the twist of yarns is made possible thereby.

15 Claims, 2 Drawing Sheets



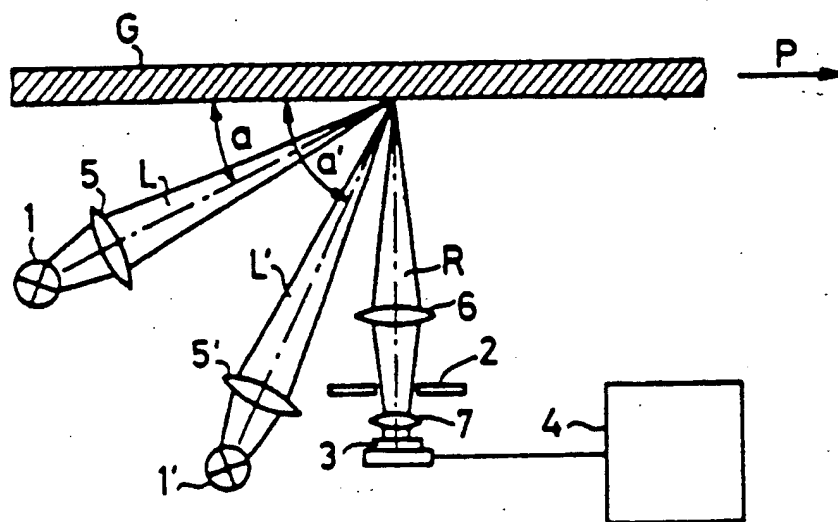


FIG. 1A

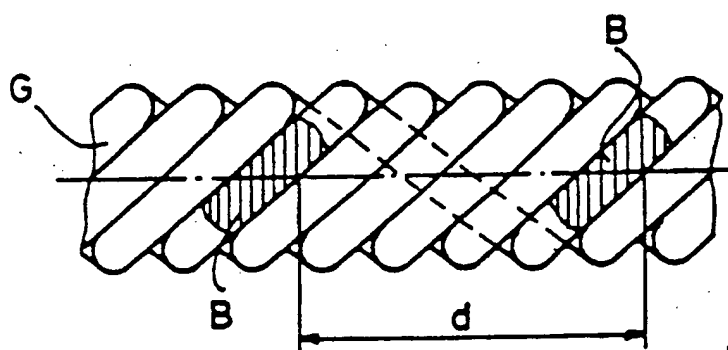


FIG. 2

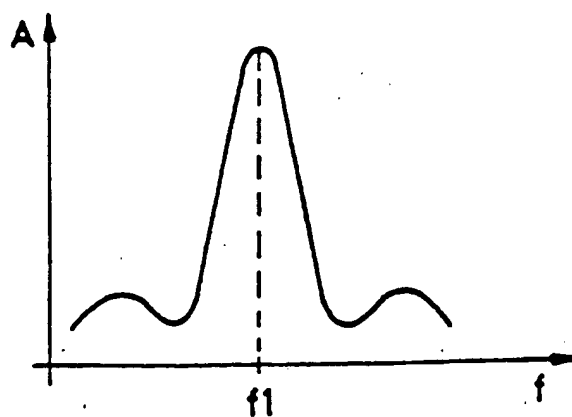
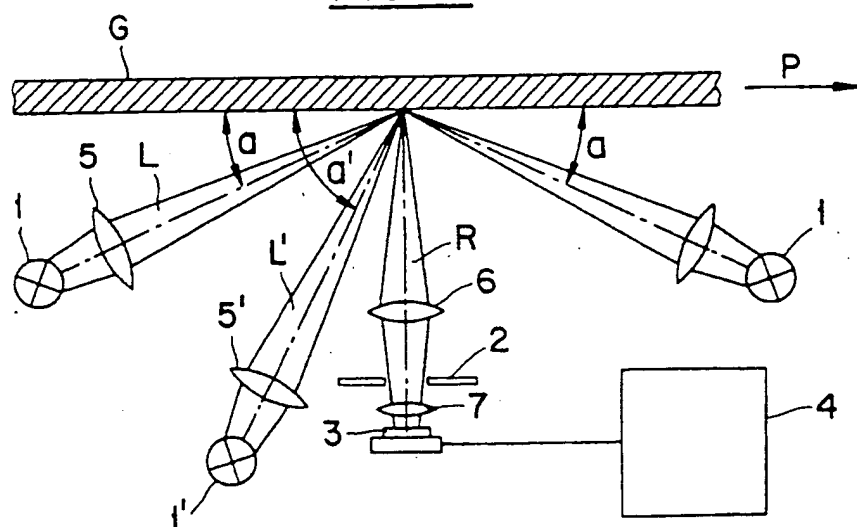


FIG. 3

FIG. 1B



PROCESS AND DEVICE FOR MEASURING THE TWIST OF A RUNNING, ELONGATE TEST BODY

BACKGROUND OF THE INVENTION

The invention relates to a process for measuring the twist of a running, elongate test body, such as, for example, of a yarn or of a wire rope, by optical scanning of its surface and analysis of the scanning signal obtained in this case.

In a process of this type which is known from DE-A-3,628,654, a thread is grazingly acted upon by light, and specifically in such a manner that a part of the light beam is shaded off by thread and the part permitted to pass by the thread impinges on a light receiver. As a result of this, it is possible to detect certain structural alterations which are a measure of the thread period. Since this process, in which the profile is scanned to a certain extent, can be employed only when a detectable profile is present at all, this process is not suitable for the measurement of the twist of yarns.

A process of the initially mentioned type for the measurement of the twist of yarns is to be indicated by the invention.

SUMMARY OF THE INVENTION

According to the invention, this object is achieved in that the test body is illuminated by at least one light source and the light reflected by the test body is imaged onto a diaphragm and is measured by at least one photoelectric receiver, the output signal of which is investigated for periodicities caused by irregularities included in the test body, and in that the twist is derived from the wavelength or from the frequency of these periodicities.

Thus, the invention proceeds from the novel finding that the twist of yarns and the like leads to the binding of irregularities, which occur periodically on account of the twist. If the surface of a continuous test body is investigated for such periodicities, then the twist can be determined from these; this would not be possible by a simple scanning of the yarn profile.

The invention further relates to a device for carrying out the process according to the invention. This device is characterized by at least one light source for illuminating the test body, a diaphragm, an optical system for imaging the light reflected by the test body onto this diaphragm, at least one photoelectric receiver disposed in the beam path downstream of the diaphragm and an evaluation unit associated with this photoelectric receiver.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention is explained in greater detail herein below with reference to an illustrative embodiment in the drawings; in the drawings:

FIG. 1A shows a diagrammatic representation of a device according to the invention,

FIG. 1B shows yet another preferred embodiment of the present invention, and

FIGS. 2,3 show diagrams for explaining the operation.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

FIG. 1A shows a piece of a yarn G, which is conveyed by guide and transport means (not shown) in the direction of the arrow P through a device for measuring

the yarn twist. As represented, this measuring device includes two light sources 1, 1' for illuminating the yarn G with a respective beam L, L', a diaphragm 2, onto which the beam R reflected by the yarn is imaged, a photoelectric receiver 3 disposed in the beam path downstream of the diaphragm and an evaluation unit 4 associated with this photoelectric receiver. Respective appropriate optical systems 5, 5' and 6 are provided in the beam path of the two beams L, L' and of the reflected beam R. A further lens 7 is situated between diaphragm 2 and photoelectric receiver 3. The light sources 1, 1' are preferably formed by light-emitting diodes, so-called LEDs.

Before the measuring device is described in greater detail, the measurement process is to be explained now with reference to FIGS. 2 and 3: any yarn G exhibiting a twist or also any wire rope or any rope and the like exhibits, on account of the twist, certain irregularities with respect to its cross-section, and specifically, in particular, deviations from the cross-sectional shape. These characteristic deviations occur periodically, in which case the spacing between two successive such irregularities or, in other words, the length of the period thereof, represents a direct measure of the twist. This is so because the yarn twisted exactly once through 360° over such a period.

If the yarn G is illuminated at a small angle to its longitudinal axis, then the said irregularities become clearly visible as bright or dark positions, as is indicated in FIG. 2 by the hatched regions B. The length of the period, or, in other words, the wavelength of the irregularities, is designated by d. If T designates the twist of the yarn as the number of turns per unit length, then the following is applicable for the period $d:d=1/T$. Customary values for T are, for example, between 300 and 1,500 turns per meter. In the case of n-fold threads, the principal period occurs at $d'=d/n$, where here d designates the period of the thread twist; thus, for example in the case of 2-fold threads, the principal period is to be expected at $d'=d/2$.

The brightness of the impinging light beam R on the photoelectric receiver 3 (FIG. 1A) then increases periodically, and if the output signal of the photoelectric receiver 3 is evaluated in the evaluation unit 4 by Fourier transformation (FFT) or autocorrelation, a clearly detectable maximum is obtained at the period of the irregularities. In the case of very well defined structures, such as, for example, in the case of wire threads or filamentary threads, this analysis can, in certain circumstances, even take place by simple determination of a trigger threshold and subsequent counting.

The analysis by means of Fourier transformation is diagrammatically represented in FIG. 3; in this case, the frequency f is plotted on the abscissa and the amplitude A on the ordinate. A clearly detectable maximum is obtained at a specified frequency f_1 ; in this case, the following then applies for the twist; $T=f_1/v$, if v designates the draw-off speed of the yarn G. In the case of analysis by means of autocorrelation, the length d of the period is obtained directly.

In the case of yarns with twist, cross-sectional fluctuations also virtually always occur, which, however, do not necessarily lead to irregularities which are periodic, i.e. evaluable for the determination of the twist. Accordingly, it is advantageous to compensate the cross-sectional fluctuations; this takes place, as represented in FIG. 1A, by the use of two light sources 1 and 1', which

illuminate the yarn G at differing angles of incidence. The angle of incidence α of the beam L is relatively small and is between 5° and 40° , preferably 5° , and the angle of incidence α' of the beam L' is steeper and is between 60° and 85° , preferably 85° .

If the two light sources L and L' are differently modulated, then, with corresponding demodulation of the signal, a single common photoelectric receiver 3 can be employed; in this case, the signal component originating from the light source 1 is divided, in this, by that originating from the light source 1'. However, it is also possible to use two light sources 1, 1', which emit light of differing wavelengths. In this case, the reflected beam R must be divided on the receiver side, and the individual components originating from the two light sources 1, 1' must be distributed by appropriate filters to two different photoelectric receivers.

A further illumination variant consists in using two light sources 1 with a small and a light source with a very steep angle of incidence of up to about 90° ; in this case, in relation to FIG. 1B the two light sources 1 are disposed symmetrically on both sides of the reflected beam R and the third light source is situated between the other two. This arrangement leads, on the one hand, to an even better emphasising of periodic structures and, on the other hand, to the elimination of disturbing influences originating, for example, from neps and the like.

The following conditions are applicable to the diaphragm 2: if the yarn exhibits T twists per unit length, and is imaged onto the diaphragm 2 at the magnification K:1, then the diaphragm 2 must be narrower than K/T in the direction of the length of the yarn, in order that it should still be possible to achieve good detection of periodic components of the twist. In the case of yarns, in the transverse dimension the diaphragm 2 is advantageously restricted to the approximate detection of the yarn body, so that the hairiness does not have an excessively disturbing effect. In order to achieve further reduction of the disturbing influences of the hairiness, the yarn G can be singed. If the yarn G is very strongly singed, then it is possible to obtain insights into the twist in the yarn body; this can, on the one hand, be very much desired in the case of rotor yarns, but is, on the other hand, destructive and should therefore be restricted to random samples.

Finally, it should furthermore be mentioned that the test body G should be guided in a vibration-free manner as far as possible, since with light incident at a small angle vibrations have a disturbing effect. For this reason, it is advantageous to guide the test body G directly at the measurement position via a deflecting component.

The described measurement device can be constructed in a very compact manner, and is therefore outstandingly suitable for use as measurement module in a device for the automatic determination of characteristic quantities of textile test material, as is described, for example, in Swiss Patent Application No. 02,823/86-2, and is known under the designation USTER TESTER (USTER—registered trade mark of Zellweger Uster AG).

What is claimed is:

1. Process for measuring the twist of a running, elongate test material comprising the steps of:
 - optically scanning a surface of the test material with at least one light source;

imaging light from said at least one light source which has been reflected by the test material onto a diaphragm;

measuring said reflected light downstream of said diaphragm using at least one photoelectric receiver said at least one photoelectric receiver producing an output signal related to said measured reflected light;

analyzing the output signal for periodic components caused by irregularities present in the test material; and

deriving twist of the test material using the wavelength or frequency of said periodic components.

2. Process according to claim 1, wherein said step of optically scanning includes a step of illuminating the test material via first and second light sources oriented at first and second angles of incidence relative to said test material, respectively.

3. Process according to claim 2, wherein said first and second light sources are differently modulated, said reflected light is measured by a single, common photoelectric receiver, and said step of measuring further includes a step of separating reflected light from said first light source and reflected light from said second light source.

4. Process according to claim 2, wherein said first and second light sources produce light of different wavelengths relative to one another, individual components of said reflected light associated with said first and second light sources being distributed to first and second photoelectric receivers, respectively, and said step of measuring further includes a step of separating reflected light received by said first photoelectric receiver and reflected light received by said second photoelectric receiver.

5. Process according to claim 1, wherein said step of optically scanning includes a step of illuminating the test material via first, second and third light sources, said first light source being oriented at a first angle of incidence relative to said test material, and said second and third light sources being disposed symmetrically relative to a beam of the reflected light and being oriented at a second angle of incidence relative to said test material, said second angle of incidence being substantially smaller than said first angle of incidence.

6. Process according to claim 1, wherein said step of analyzing includes a step of evaluating said output signal using a Fourier analysis.

7. Process according to claim 1, wherein said step of analyzing includes a step of evaluating said output signal using autocorrelation.

8. Process according to claim 1, wherein said elongate test material is yarn.

9. Process according to claim 1, wherein said elongate test material is a wire rope.

10. Apparatus for measuring the rotation of a running, elongate test material comprising:

at least one light source for optically scanning a surface of a test material;

an optical system for imaging light from said at least one light source which has been reflected by said test material onto a diaphragm;

at least one photoelectric receiver disposed in a light beam path downstream of said diaphragm for producing an output signal related to said reflected light; and,

means for analyzing said output signal to detect periodic components caused by irregularities present in

the test material and for deriving a rotation of the test material from the wavelength or frequency of said periodic components.

11. Apparatus according to claim 10, wherein said at least one light source illuminates said test material at an oblique angle relative to direction of travel of the test material.

12. Apparatus according to claim 10, wherein said at least one light source includes first and second light sources oriented at first and second angles of incidence relative to said test material, said first angle being smaller than said second angle.

13. Apparatus according to claim 10, wherein said diaphragm has a dimension in a longitudinal direction of

said test material which is less than a value K/T , where T corresponds to a number of rotations of the test material per unit length and the test material is imaged onto the diaphragm with a magnification factor $K:1$.

14. Apparatus according to claim 13, wherein said diaphragm has a dimension in a transverse direction of said test material such that light reflected by fibrous elements projecting from a surface of the test material is substantially prevented from reaching said at least one photoelectric receiver.

15. Apparatus according to claim 18, wherein a deflecting component is provided to guide the test material toward a measurement position.

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